CASE REPORT

A case series highlighting a common approach to identifying anti-Jk3

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The Kidd-null phenotype, Jk(a–b–), is rare, and a patient with this phenotype may develop anti-Jk3, a red blood cell (RBC) antibody reactive with a domain common to both Jk^a and Jk^b. Like other antibodies to high-prevalence antigens, the presence of this antibody poses challenges in the immunohematologic evaluation of these patients. Thoughtful laboratory testing is necessary to resolve the antibody specificity and to reveal other underlying antibodies. Moreover, the rarity of the Kidd-null phenotype makes finding blood donors difficult for those who need transfusion and have developed anti-Jk3. This review describes methods used in identifying anti-Jk3 in four pregnant patients. Blood bank records were retrospectively reviewed to illustrate the common approach in anti-Jk3 identification. In all cases, pertinent blood bank history was gathered, and extended RBC phenotyping was performed, followed by adsorption studies and testing of selected RBCs. Underlying antibodies were found in two of the cases. This review also reiterates some common challenges encountered with Kidd antibody analysis and highlights the importance of patient ethnic ancestry and obtaining accurate patient transfusion history. ***Immunohematology*** **2021;37:84–88. DOI:10.21307/immunohematology-2021-013.**

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Identifying an antibody against a high-prevalence antigen is a challenging aspect of transfusion medicine. To narrow the possible cause of reactivity, knowledge of the patient's ethnicity, transfusion history, and pregnancy history are key elements.[1] Moreover, known properties of the antibody such as common phase of reactivity and reactivity against chemical- and enzyme-treated red blood cells (RBCs) as well as the availability of rare RBCs are important factors used to identify the antibody.[1] In addition, testing the patient's RBCs using antisera to high-prevalence antigens and RBC genotyping can aid in confirming the antibody specificity.[1]

Some antibodies to high-prevalence antigens may be suspected based on the patient's ethnicity, such as anti-Fy3, anti-U, and anti-Js^b among individuals of African descent; anti-Kp^b and anti-k among individuals of European descent; anti-H among individuals from the Indian subcontinent; anti-Di^b among those of Asian, South American Indian, and Native American descent; and anti-Jk3 among individuals of Finnish, Asian, and Polynesian descent.[1,2]

The Kidd system was first described in the 1950s, and there are currently four known phenotypes: Jk(a–b+), Jk(a+b–), Jk(a+b+), and Jk(a–b–).[3] The Jk(a–b–) phenotype was first described by Pinkerton et al.[4] in a Filipina individual in 1959. Jk(a–b–) is uncommon among individuals of European descent ($p < 0.01$) and is more common among individuals of Finnish, Asian, and Polynesian descent.[1,2,3,5] Antibodies in this system are known to cause acute and delayed transfusion reactions, but only rarely cause severe hemolytic disease of the fetus and newborn (HDFN).[3,5] They are usually of immunoglobulin (Ig)G1 and IgG3 types and often cause hemolysis by activating complement.[3,5] Anti-Jk3 reacts with all Kidd phenotypes except Jk(a–b–).[3,5] This report presents four cases of pregnant women who developed anti-Jk3.

Case Presentation

Case 1

A 27-year-old G3P2 Polynesian woman, with a history of transfusion 1 year previously, presented for prenatal care. Her sample was referred to the American Red Cross Reference Laboratory for antibody identification. The patient's plasma reacted with all RBCs tested in polyethylene glycol (PEG) by the indirect antiglobulin test (IAT); however, the autocontrol and her polyspecific direct antiglobulin test (DAT) were negative. Blood typing and extended phenotyping were also performed. Her RBCs tested as group A, D+C+E–c–e+; K–; Fy(a+b–); Jk(a–b–); Le(a+b–); P1+; and M+N+S–s+. Based on the reactivity of her plasma in conjunction with her Jk(a–b–) phenotype, anti-Jk3 was suspected. The laboratory then tested her plasma against rare Jk(a–b–) RBCs. These RBCs were nonreactive with the patient's plasma. To determine if there were any underlying antibodies, warm adsorptions with papain-treated, phenotypically similar RBCs were performed. Two sets of adsorptions were performed: one with Jk(a–) RBCs and one with Jk(b–) RBCs. The plasma adsorbed with the Jk(a–) RBCs revealed an underlying anti-Jk^a. All other common clinically significant antibodies were ruled out using Jk(a–b–) RBCs, adsorbed plasma, and the patient's phenotype.

Antibody titration using Jk(a+b+) RBCs was also performed. The combined titer of anti-Jk^a and anti-Jk3 was 16 with a score of 45. The sample was not sent for RBC genotyping.

Case 2

A 24-year-old G3P2 woman of unknown ethnicity was admitted for delivery. Her transfusion history was also unknown. The patient's plasma reacted with all RBCs tested, which prompted the hospital to send a sample to the American Red Cross Reference Laboratory for evaluation. Testing revealed the patient's RBCs to be group A, D+C+E−c−e+; K−; Fy(a+b−); Jk(a−b−); Le(a+b−); and M+N−S−s+. The patient's plasma reacted w+ to 1+ in low-ionic-strength saline (LISS)-IAT and 1+ to 2+ in PEG-IAT. The autocontrol was nonreactive, and the polyspecific DAT was negative. Anti-Jk3 was suspected based on the patient's Jk(a−b−) phenotype and the reactivity observed in her plasma. Her plasma was then tested against Jk(a−b−) RBCs from the laboratory's frozen inventory and found nonreactive, which confirmed the presence of anti-Jk3. To further evaluate for other alloantibodies, warm adsorptions with phenotypically similar papain-treated RBCs were performed to remove the anti-Jk3. The laboratory used Jk(a−b+) adsorbing RBCs and tested the adsorbed plasma with selected Jk(a−b+) RBCs. Other common clinically significant antibodies were ruled out using the adsorbed plasma and the patient's phenotype. The presence of separable anti-Jk^a and anti-Jk^b was not evaluated in this case. Antibody titration and RBC genotyping were not performed.

Case 3

Reactivity with all RBCs was observed in the testing of the plasma of a 33-year-old Asian G2P1 woman at 39 weeks' age of gestation on her prenatal care visit. The sample was then submitted to the American Red Cross Reference Laboratory for further evaluation. The patient had never been transfused, and her hemoglobin (Hgb) at that visit was 12.2 g/dL (normal range 11.1–15.9 g/dL). Similar to the cases described earlier, the autocontrol was nonreactive, and the DATs tested by gel and tube methods using polyspecific antihuman globulin (AHG) were negative. This reactivity pointed to a possible antibody to a high-prevalence antigen. To gain further information in the investigation, the patient's blood type and extended phenotype were determined. Her RBCs tested as group A, D+C+E+c+e+; K−; Fy(a+b−); Jk(a−b−); and S−s+. The reference laboratory concluded that it was a probable anti-Jk3 based on the patient's Jk(a−b−) phenotype and ethnicity. The reference laboratory did not have Jk(a−b−) RBCs available to further confirm the specificity, and the antibody was thus identified as "probable anti-Jk3." To facilitate the removal of the probable anti-Jk3 to allow for further antibody exclusions, warm adsorptions were performed. Two sets of papain-treated RBC stroma were used: R_1R_1; K−; Jk(a−), and R_2R_2; K−; Jk(b−). No underlying antibodies were observed in the adsorbed plasma. The patient's sample was sent for molecular testing, which revealed a genotype of *JK*02N.01/JK*02N.01* with a predicted phenotype of Jk(a−b−).

Case 4

A 35-year-old G1P0 Asian woman was admitted for induction of labor. The patient's transfusion history was unknown, and her Hgb was 4.4 g/dL (normal reference range unknown) at the time of admission. Evaluation of the patient was referred to the American Red Cross Reference Laboratory because of panreactivity by the IAT with a nonreactive autocontrol. Further testing revealed the patient's RBCs had a negative DAT (gel/IgG and tube/polyspecific AHG) and tested as group A, D+C+E−c−e+; K−; Fy(a+b−); Jk(a−b−); and S−s+. Based on the patient's Jk(a−b−) phenotype and plasma reactivity, anti-Jk3 was suspected, but the plasma was reactive with two group O rr Jk(a−b−) RBCs. The plasma was also differentially warm adsorbed with R_1R_1 Jk(a−b+), R_2R_2 Jk(a−b+), and rr Jk(a+b−) papain-treated RBC stroma to remove the anti-Jk3. Warm adsorptions revealed anti-Jk^b in the adsorbed plasma using the gel method. Because of reactivity with Jk(a−b−) RBCs, the sample was sent to another reference laboratory for further investigation. The second reference laboratory found four additional Jk(a−b−) RBCs nonreactive with the patient's plasma by LISS-IAT and PEG-IAT techniques. Because the patient's RBCs tested as R_1R_1 and had reactivity with rr Jk(a−b−), anti-c was suspected but ruled out when the R_1R_1-adsorbed plasma tested against R_2R_2 RBCs was nonreactive; a probable anti-f was then identified. Not all common alloantibodies could be excluded using the Jk(a−b−) RBCs, and, thus, differential adsorption studies with ZZAP (DTT-ficin)-modified allogeneic RBCs were performed to intentionally adsorb out the anti-Jk3. The adsorbed plasma revealed anti-Jk^a and anti-Jk^b by LISS-IAT. Lastly, the second reference laboratory also identified an anti-P1 at room temperature with some reactivity carried through the IAT. The patient's plasma reacted with most P1+ Jk(a−b−) RBCs at room temperature but was nonreactive at 37°C and by the IAT using a strict prewarm technique.

Materials and Methods

In all cases, antibody identification was performed using PEG-IAT, and DATs were performed in tube using polyspecific AHG. In addition, case 2 was tested in LISS-IAT, case 3 was tested using IgG ID-MTS Gel (Ortho Clinical Diagnostics, Raritan, NJ), and case 4 was tested using IgG ID-MTS Gel (Ortho Clinical Diagnostics) and LISS-IAT. Additional DATs in cases 3 and 4 were performed using IgG ID-MTS Gel. The titration performed in case 1 was tested in saline-IAT for 30 minutes. A Precise Type HEA Molecular BeadChip kit (Immucor, Norcross, GA) was used to perform the molecular testing in case 3. Adsorption studies in cases 1 and 2 used papain-treated, allogeneic, phenotypically similar RBCs, and cases 3 and 4 used papain-treated, allogeneic RBC stroma. Additional adsorption studies performed in case 4 used ZZAP (DTT-ficin)-treated allogeneic RBCs.

Discussion

Kidd antibodies are immunogenic and notoriously known to weakly react by the IAT.[3,5] Although strong reactivity with test RBCs can be seen, it is not uncommon to find Kidd antibodies reacting more strongly with RBCs carrying a double dose of Jk^a or Jk^b than with single-dose antigen-positive RBCs.[3] Using enzyme-treated RBCs can strengthen the reactivity of Kidd antibodies.[3,5] The possible cause of weak reactivity of the Kidd antibody in the methods used is that most test modalities now use plasma for testing and anti-IgG in the IAT. Kidd antibodies can react more strongly when using polyspecific AHG containing anti-C3.[3] In addition, it is known that Kidd antibody titers quickly decline, and the antibody can become serologically non-demonstrable a few weeks to months after initial identification.[3,5] Because of their unreliable detectability *in vitro,* it is not surprising that Kidd antibodies are a common cause of delayed hemolytic transfusion reactions.[3,5] It is therefore important to inform patients about their immunization to Kidd antigens and encourage them to mention this as part of their past medical history, especially if blood transfusion is indicated.

Antibodies to high-prevalence antigens typically present with panreactivity of equal strength, a nonreactive autocontrol, and a negative DAT.[1] This typical reactivity was seen in all four cases described herein, but it is also possible for antibodies to high-prevalence antigens to present with a positive DAT and/or autocontrol when other clinical scenarios are present. A case reported by McCaskill et al.[6] described a Filipino man with chronic obstructive pulmonary disease, hypertension, hyperlipidemia, and drug-induced hepatitis whose plasma reacted with all test RBCs including his autocontrol. The patient's DAT was also positive. After further testing, it was concluded that the patient had anti-Jk3, and the positive DAT was possibly due to his recent transfusion.[6]

The initial approach in all four cases was to determine the extended phenotype and gather pertinent blood bank information about the patient. Table 1 contains a summary of the patients' blood bank history and the laboratory results pertinent to these case studies.

Table 1. Summary of pertinent patient blood bank history and laboratory results

Parameter	Case 1	Case 2	Case 3	Case 4
Ethnicity	Polynesian	Unknown	Asian	Asian
Transfusion history	1 year ago	Unknown	Never been transfused	Unknown
Obstetric score	G3P2	G3P2	G2P1	G1P0
Known RBC antibodies	None	None	None	None
Hemoglobin (g/dL)	Unknown	Unknown	12.2	4.4
ABO/D	A, D+	A, D+	A, D+	A , D+
Extended phenotype	R_1R_1; K–; Fy(a+b–); Jk(a–b–); M+ N+ S– s+	R_1R_1; K–; Fy(a+b–); Jk(a–b–); M+ N– S– s+	R_1R_2; K–; Fy(a+b–); Jk(a–b–); S– s+	R_1R_1; K–; Fy(a+b–); Jk(a–b–); S– s+
DAT	Negative	Negative	Negative	Negative
Antibody identification	Anti-Jk3, anti-Jk^a	Anti-Jk3	Probable anti-Jk3	Anti-Jk3, anti-Jk^a, anti-Jk^b, probable anti-f, anti-P1
Titer	16	Not performed	Not performed	Not performed
RBC genotyping	Not performed	Not performed	*JK*02N.01/JK*02N.01*	Not performed

RBC = red blood cell; DAT = direct antiglobulin test.

All patients demonstrated a Kidd-null phenotype, Jk(a–b–), and therefore were suspected to have anti-Jk3. To confirm the presence of anti-Jk3, the plasmas in all cases except case 3 were tested with rare Jk(a–b–) RBCs and found to be nonreactive. To facilitate removal of the anti-Jk3, adsorption studies were performed in all cases. Cases 2 and 3 had no underlying antibodies, whereas case 1 was found to have an underlying anti-Jk^a, and case 4 had underlying probable anti-f, anti-Jk^a, anti-Jk^b, and anti-P1. Only the sample in case 3 was sent for RBC genotyping.

Titration studies with RBCs carrying a single-dose antigen were performed in case 1. HDFN due to Kidd antibodies is often mild and may be because newborns have fewer complement components compared with adults and therefore less complement interaction with the antibodies.[3] A case study by Lawicki et al.[7] described the clinical severity of HDFN in four cases of pregnant women with alloanti-Jk3. In two of the cases, the HDFN was of mild to moderate severity, while the other two had no clinically significant HDFN.[7] The case of moderate HDFN severity had a maximum anti-Jk3 titer of 128. However, one of the clinically insignificant cases had a higher titer compared with that of the mildly severe HDFN case (32 vs. 16).[7] This study further established that the significance of anti-Jk3 titer remains unclear. Moreover, because of a lack of data in the literature that correlate anti-Jk3 titers and severity of HDFN, it is reasonable to monitor pregnant patients who develop anti-Jk3 for possible HDFN.[7]

In case 2, anti-Jk^a and anti-Jk^b were not ruled out. It is not necessary to rule out the related specificities in patients with an antibody against the high-prevalence antigen lacking in a null phenotype. Moreover, the transfusion recommendation is not dependent on whether these antibodies are present. Case 3 was evaluated in a reference laboratory that did not have rare RBCs lacking high-prevalence antigens available for testing. The presence of anti-Jk3 was not confirmed by demonstrating her plasma was nonreactive with Jk_{null} RBCs and was therefore called probable anti-Jk3. The antibody specificity was based on the plasma reactivity and patient's phenotype. The alleles found in the genotyping in this case are seen among individuals of Polynesian descent. There is a splice site mutation in intron 4 of the null allele that results in an absence of the JK protein from the membrane.[8]

Removal of anti-Jk3 by adsorption using either Jk(a+b–), Jk(a–b+), or Jk(a+b+) RBCs or RBC stroma was performed to evaluate other concomitant antibodies possibly present with the anti-Jk3. Case 4 is a good example of anti-Jk3 with underlying antibodies. Despite the suspicion of anti-Jk3, the initial testing with Jk(a–b–) RBCs was positive. This finding indicated that another antibody may be present or that the reaction may not be due to anti-Jk3. Looking closely at the rare RBCs tested in the initial reference laboratory revealed that the two Jk(a–b–) RBCs used were group O, rr (C–E–c+e+f+). f is a compound antigen expressing c and e in cis formation. f is absent when c and e are found on two different proteins (*trans* position) such as R_1R_2 (DCe/DcE) or when c and e are not seen together such as R_1R_1 (DCe/DCe) or R_2R_2 (DcE/DcE).[9] Based on the patient's phenotype in case 4, her RBCs are f–, and thus she can develop anti-f. Because of multiple unexpected reactivities and lack of additional rare Jk(a–b–) RBCs to confirm anti-Jk3, another sample was collected and referred to a second reference laboratory. This laboratory tested four Jk(a–b–) RBCs. These RBCs were nonreactive, which confirmed the presence of anti-Jk3. They also found that anti-Jk^a and anti-Jk^b were present after removing anti-Jk3 by adsorption. It is worth mentioning that anti-Jk^a was not detected by the initial reference laboratory, and this is most likely due to the known variable reactivity of Kidd antibodies *in vitro* and the possibility of these antibodies being undetected when using IgG ID-MTS Gel. In 1996, a study by de Castilho et al.[10] compared the sensitivity of serologic methods in detection of antibodies. They found that in reference samples, PEG-IAT was the most sensitive method for detection of Kidd antibodies. However, in the same study, it was found that gel LISS testing was a more sensitive modality in detecting antibodies for routine samples. Another study done by Kay et al.[11] concluded that solid-phase red cell adherence (SPRCA) was more sensitive in detecting Kidd antibodies than IgG ID-MTS Gel. They noted a statistically significant increase in rate of anti-Jk^a detection from 5.4 to 11.86 per 10,000 solid-phase antibody screens performed when they switched from IgG ID-MTS Gel to SPRCA as a primary method for antibody screening. Considering that Kidd antibodies are more easily detected in PEG-IAT or LISS-IAT, the second reference laboratory was most likely to detect the anti-Jk^a because they used LISS-IAT when testing their adsorbed plasma.

Although none of the cases used the urea lysis test, this laboratory test can aid in identification of the Jk(a–b–) phenotype, particularly in large-scale donor testing.[6] The Jk_{null} phenotype is known to be resistant to urea-induced lysis.[3,5,6,12] RBCs with other Kidd phenotypes will lyse in the presence of 2 M urea.[6,12]

The patients in this report did not require RBC transfusions. The clinical status and hematocrit of these patients remained stable throughout their hospital stay.

Conclusion

A high-prevalence antigen is an antigen present in almost all populations, and its absence is considered rare (<1:1000).[1] This fact makes finding blood for a patient who develops an antibody against a high-prevalence antigen difficult. Stable healthy patients who have an antibody to a high-prevalence antigen are encouraged to donate autologous units.[1,7] If the condition of the patient does not allow this type of donation, testing their family members for the phenotype needed is another option.[1,7] In addition, the American Rare Donor Program is a network used by reference laboratories to aid in finding rare blood units.[1,7]

Anti-Jk3 is an example of an antibody against a high-prevalence antigen. Because of the continuous growth and diversity of the U.S. population, it is important to remember its prevalence among certain ethnicities. Donor recruitment geared towards individuals in these ethnic populations can increase the inventory of Jk_{null} RBC units.

When dealing with reactivity consistent with an antibody to a high-prevalence antigen, obtaining history regarding transfusion, pregnancy, and patient's ethnicity are the first steps to narrowing the possible cause of reactivity.[1] Therefore, obtaining an accurate and complete blood bank history is of utmost importance.[3] An extended phenotype including high-prevalence antigens that are commonly lacking in individuals in the ethnic group of the patient is helpful in further narrowing the possible cause of reactivity.[1] Testing RBCs after enzyme treatment with papain or ficin, chemical treatment with DTT or EDTA glycine acid, and adsorption studies can also help pinpoint the specificity of the antibody.[1] By carefully analyzing the pattern of reactivity observed, selected rare RBCs can then be tested to confirm the presence of the antibody.

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